OBSERVATIONS ON CHANGES IN VIRULENCE OF HEMOLYTIC STREPTOCOCCI WITH SPECIAL REFERENCE TO IMMUNE REACTIONS

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In the following pages are recorded the results of observations on changes in virulence of hemolytic streptococci produced by animal passage, growth in artificial culture, and certain other conditions. At the same time the reactions with immune serums of streptococcal strains of varying degrees of virulence were studied and the results thus obtained are stated briefly.

EXPERIMENTS ON VIRULENCE OF STREPTOCOCCI

Careful observations were made on the changes in virulence of a streptococcus as it was grown on blood agar. This strain was isolated from a pleural empyema and in the first culture in ascites broth 0.05 c c of a 24-hour growth killed a mouse within 48 hours. The coccus was not taken up by g. pig leukocytes in the presence of normal serum; formed long chains in broth; was facultatively anaerobic, and of the beta hemolytic type. It was passed through 10 mice in succession, and in order to test the virulence, it was now grown from the heart blood and inoculated as follows: The heart was dropped into a tube with 2 c c of salt solution, the tube shaken, then centrifugated, and 0.2 c c of the supernatant fluid after being diluted several times with salt solution was injected into the peritoneal cavity of a mouse, the same quantity being plated on dextrose agar in order to get some idea of the number of bacteria present. Cultures were made in broth one part and inactivated goat serum two parts and in 24 hours at 37 C., after being diluted in salt solution, 0.2 c c was injected into the peritoneal cavity of a mouse, and the same quantity plated on dextrose agar. The suspension from which the cultures were made were first shaken in a tube with small glass balls in order to break up the chains. Similar experiments were made with cultures directly from the heart on 5% goat blood agar, using the bacteria growing above the water of condensation and suspending them in salt solution. The results, which are given in table 1, show that the virulence is reduced at once in artificial culture. Thus 46 streptococci from the heart blood of a mouse killed by the same organism were sufficient to kill, but when grown in serum broth a larger number of cocci were required to kill and when grown on blood agar a still larger number. Apparently serum broth is a much more favorable medium for the conservation of the original virulence than blood agar.

Apparently something in the culture medium reduced the virulence, and the influence of peptone was studied. Streptococci were grown for 24 hours at 37 C. on goat blood agar with 5% and 15% of peptone and without any peptone, and the virulence tested by peritoneal injections in mice, the number of cocci

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being determined as before by the plate method. Before inoculation the culture medium was made strictly neutral. As shown in table 2, it required 84,000 streptococci cultivated on blood agar without peptone to kill a mouse within 24 hours, whereas 12,400 and 18,000 streptococci grown on 5% and 15% peptone blood agar, respectively, constituted a lethal dose. It may be concluded that peptone is not a chief factor in reducing virulence.

TABLE 1

THE EFFECT OF CULTURE ON THE VIRULENCE OF THE STREPTOCOCCUS

Mice	Source and Number of Streptococci Injected and Results		
	From the Heart of a Mouse	24-Hour Serum Broth Culture	24-Hour Agar Broth Culture
1-3	37,400—died within 17 hours	106,000—died within 17 hours	300,000—died within 17 hours
4-6	37,400—died within 17 hours	106,000—died within 24 hours	300,000—died within 17 hours
7-9	3,740—died within 17 hours	10,600—died within 48 hours	30,000—died within 17 hours
10-12	3,740—died within 20 hours	10,600—died within 72 hours	30,000—died within 17 hours
13-15	280—died within 20 hours	696—died within 72 hours	3,000—died within 24 hours
16-18	280—died within 36 hours	696—died within 72 hours	3,000—died within 24 hours
19-21	46—died within 46 hours	109—survived	300—survived
22-24	46—died within 36 hours	109—survived	300—survived
25-27	46—survived	109—survived	30—survived
27-30	46—survived	109—survived	30—survived

TABLE 2

INFLUENCE OF PEPTONE IN BLOOD AGAR ON VIRULENCE OF STREPTOCOCCI

Mice	Amount of Peptone in Blood Agar, Number of Streptococci Injected and Results		
	No Peptone	5% Peptone	15% Peptone
1-3	84,000—died within 17 hours	124,000—died within 17 hours	180,000—died within 17 hours
4-6	84,000—died within 17 hours	124,000—died within 17 hours	180,000—died within 17 hours
7-9	8,400—survived	12,400—died within 24 hours	18,000—died within 24 hours
10-12	8,400—survived	12,400—died within 24 hours	18,000—died within 24 hours
13-15	840—survived	1,240—survived	1,800—survived
16-18	840—survived	1,240—survived	1,800—survived
19-21	84—survived	124—survived	180—survived
22-24	84—survived	124—survived	180—survived

Next, the influence of the reaction of the medium on the virulence was tested. Turro[1] recommended acid culture medium for streptococci because they live longer and maintain their virulence better on an acid medium. I used agar with 1% peptone, 5% goat blood, and of three different reactions, namely, 0.8% acid to phenolphthalein, 0.5% alkalin, and neutral. Table 3 shows

[1] Centralbl. f. Bakteriol. I, O., 1895, 17, p. 864.

that 1,344 streptococci were killed after growth on the alkaline medium, 2,580 after growth on the neutral medium, and only 510 when grown on the acid medium. These results support the conclusion of Turro.

I then tested the influence of oxygen, the virulence being determined as before, after growth on the surface of 5% goat blood agar and anaerobically in the depths of such blood agar tubes, in each case at 37 C. for 24 hours. It was found that at least 8,500 streptococci grown aerobically were required to kill a mouse within from 36 to 48 hours and 1,100 grown anaerobically.

TABLE 3

INFLUENCE OF REACTION OF CULTURE MEDIUM ON VIRULENCE OF STREPTOCOCCI

Mice	Number of Streptococci, Reaction of Medium, and Results		
	Alkaline	Neutral	Acid
1-2	134,400—died within 24 hours	258,000—died within 17 hours	
3-5	134,400—died within 24 hours	258,000—died within 17 hours	
6-8	13,440—died within 25 hours	25,800—died within 17 hours	51,000—died within 17 hours
8-11	13,440—died within 24 hours	25,800—died within 17 hours	51,000—died within 17 hours
11-13	1,344—died within 24 hours	2,580—died within 36 hours	5,100—died within 17 hours
14-16	1,344—died within 48 hours	2,580—died within 48 hours	5,100—died within 17 hours
17-19	1,344—survived	258—survived	510—died within 36 hours
20-22	1,344—survived	258—survived	510—died within 36 hours
25	survived	survived	51—survived
25-27	.....	.....	51—survived
28-30	.....	.....	51—survived

TABLE 4

INFLUENCE OF OXYGEN TENSION ON VIRULENCE OF STREPTOCOCCI

Mice	Manner of Culture, Number of Streptococci, and Results	
	Aerobic Culture	Anaerobic Culture
1-2	85,000—died within 24 hours	110,000—died within 24 hours
3-4	85,000—died within 24 hours	110,000—died within 24 hours
5-6	8,500—died within 36 hours	11,000—died within 24 hours
7-8	8,500—died within 36 hours	11,000—died within 36 hours
9-10	850—survived	1,100—died within 36 hours
10-12	850—survived	1,100—died within 48 hours
13-14	85—survived	110—survived
15-16	85—survived	110—survived

The streptococci used in the following experiment came from a human abscess and had been cultivated for about a year. It was nonvirulent for mice and rabbits, 0.5 c c of a 24-hour serum broth culture injected into the abdomen of a mouse, and 3 c c injected intravenously in a rabbit being without effect. The coccus was strongly hemolytic on blood agar and formed long chains in broth. Intravenous injections were made into a rabbit and the coccus was grown from the heart blood in one part of broth and one part of inactivated rabbit serum (56 C. for 30 minutes). This process was repeated five times, and after the fifth passage 2 rabbits were injected with 0.2 c c per

kilo of a 24-hour serum broth culture, 2 others with 0.1 c c per kilo of the same culture. The rabbits that received 0.2 per kilo died within 3 days while the rabbits that received only 0.1 c c developed diarrhea and became thin. The virulence of the coccus for mice had now increased so that 0.05 c c of the culture used in the injection of the rabbit killed within 24-48 hours, and the streptococcus was now passed in the heart blood through 18 mice in succession, and the virulence tested. It was found that 0.00001 c c of a 24-hour serum broth culture killed within 48 hours. Six rabbits were now injected; 2 received intravenously 0.01 c c of serum broth culture per kilo, but no special effect was noticed; 2 received 0.05 c c per kilo, one dying within 48 hours, the other within 4 days; in the remaining two 0.1 c c per kilo was injected and one died within 36 hours, the other within 4 days. The coccus was then passed through 24 mice, the virulence was tested again and found to be the same as when it had passed through 18 mice, 0.00001 c c of the serum broth culture killing within 48 hours.

TABLE 5

VIRULENCE TEST OF STREPTOCOCCUS AFTER PASSAGES THROUGH MICE

Number of Animal	Quantity of Culture	Results
1	0.001	Died within 17 hours
2	0.001	Died within 17 hours
3	0.0001	Died within 48 hours
4	0.0001	Died within 48 hours
5	0.00001	Died within 48 hours
6	0.00001	Died within 48 hours
7	0.000001	Survived
8	0.000001	Survived
9	.....	Survived
10	.....	Survived

Streptococci in the heart blood of the dead mice.

It may be concluded from these results that an avirulent hemolytic streptococcus grown artificially for some time acquires increased virulence both for rabbits and mice on being passed through rabbits, and that after such passage further passages in mice increases the virulence especially for mice, but also for rabbits; furthermore, that when a certain maximum virulence is attained by animal passage this virulence is not readily changed by further passage through the same animal.

As stated, the fatal dose for mice of the 24-hour serum broth culture of the streptococcus under consideration was 0.00001 c c after it had passed through 24 mice. For guinea-pigs 2 c c of the serum broth culture was fatal within 48 hours (minimum fatal dose not determined). Cultures were made from the heart blood in serum broth and after being passed through 7 guinea-pigs the lethal dose was determined as shown in table 3. It appears that a streptococcus 0.00001 c c of a broth culture of which kills a mouse, became so reduced in virulence after being passed through guinea-pigs that 0.01 c c was required to kill a mouse. We may say that when the maximum virulence of a streptococcus for the mouse is reached, passage through the guinea-pig increases the virulence for the guinea-pig, but decreases the virulence for the mouse. On the other hand, when the virulence for the mouse is increasing but has not attained the maximum, the passage of the streptococcus through the guinea-pig increases the virulence for both the mouse and the guinea-pig.

It has been suggested that streptococci and other bacteria of the same class form toxins when in contact with the tissues of the infected body (Lindemann, Friedberger, Neufeld and Dold, and others), but no definite information in regard to this point is at hand. I have studied the effect on streptococci in collodion sacs placed in the abdominal cavity of rabbits. Caliero[2] concluded that the virulence of streptococci was increased by sojourn in collodion sacs inserted in the abdominal cavity of guinea-pigs, but decreased for rabbits. Tuneoka[3] found that staphylococcus grew in virulence while in sacs in the cavity of rabbits previously immunized with the organism.

The collodion sacs were made in the usual way, care being taken to obtain a thin membrane so that the body fluids surely would pass through. Serum broth (1-1) cultures of streptococci, 24 hours old, were centrifugated and a loop of the sediment suspended in 1 c c of salt solution and a certain quantity introduced into each sac, one being placed in each side of the peritoneal cavity of a rabbit. The streptococcus was the one used in the previous experiment; it was not virulent for mice, rabbits or guinea-pigs, 0.5-1 c c of rabbit serum broth culture could be injected into the peritoneal cavity of a mouse without any effect.

Exper. 1.—One collodion sac with 1 c c and one with 0.5 c c of streptococcus suspension, prepared as described were left in the peritoneal cavity of a rabbit for 6 days and then were opened. One c c of salt solution was introduced into each sac and after mixing it carefully with the white contents, 1 c c was put into each of 2 freshly made sacs, which were then placed in the cavity of a rabbit. A rapid loss of weight followed and after 3 days the rabbit died from peritonitis, not due to streptococci. The sacs were intact, rabbit serum broth cultures were made of the contents, and the virulence tested for mice after 20 hours at 37 C. The lethal dose was found to be 0.3 c c, showing that the virulence had increased during the sojourn in the abdomen of the rabbit.

As I had found previously that the virulence of a streptococcus brought up to the maximum for a particular animal is reduced by passage through another animal, while during the period of increasing virulence passage through another animal may serve still further to increase the virulence for the first animal, I tried to find out whether such changes occur when streptococci are kept in a collodion sac in the abdominal cavity. For this purpose I used the streptococcus obtained from a pleural empyema and cultivated it artificially for about 6 months when it was passed through 10 mice. At the time of the experiment 0.001 c c of a 24-hour rabbit serum broth (1-1) culture was a fatal dose for mice.

Exper 2.—A 24-hour rabbit serum broth culture was centrifugated and the sediment suspended in salt solution, one loopful to 1 c c; of this suspension 1 c c was placed in each sac and 2 sacs were introduced into the abdominal cavity, but the animal died from streptococcus peritonitis, one of the sacs having broken. The intact sac was opened, 1 c c of salt solution added to the contents and 0.5 c c of this suspension were placed in each of 2 sacs which were then introduced into the abdomen of a fresh rabbit where they remained for 2 weeks, When removed there was a small amount of whitish yellow material in the sacs, which were intact, and preparation showed that many of the streptococci remained unstained. Cultures were made in rabbit serum broth and the virulence tested for mice. Before being introduced into the abdomen, the lethal dose was 0.001 c c of a 24-hour serum broth culture; after having been kept in the abdomen for 16 days, the fatal dose was the same. Apparently no change in virulence for mice had taken place.

EXPERIMENTS ON AGGLUTINABILITY OF STREPTOCOCCUS

The question whether the agglutinability of a streptococcus strain changes under different conditions is an important one from the point of view of the grouping of streptococci. As pointed out previously,[4] the use of cinnobar obviates the action of minor agglutinin, at least to some extent, and I have found further that cinnobar also prevents spontaneous agglutination of streptococci. Twenty-four hour cultures of streptococci in 0.2% dextrose broth were centrifugated, the bacteria washed with salt solution, and a suspension made to

[2] Centralbl. f. Bakteriol. I, O, 1914, 47, p. 208.

[3] Nippon Biseibutsu Gakkan Zasshi, 1916, 3, p. 382.

[4] Nakayama, Jour. Infect. Dis., 1919, 24, p. 489.

which a small quantity of cinnobar was added. The tubes were then shaken thoroughly and left to stand for 2 or 3 hours when they were centrifugated for a little while until a homogenous suspension was produced. This treatment not only breaks up the chains, but seems to make the cocci less sensitive to agglutinin. The agglutinating serum was prepared by injecting rabbits with streptococci: twenty-four-hour dextrose broth cultures were centrifugated, the sediment suspended in salt solution and after being heated at 60 C. for one hour, injected intravenously. Three or more injections were then given of the same material, but without being heated, at intervals of 5-7 days, and serum obtained 7-10 days after the last injection.

I now studied the changes in agglutinability of a streptococcus on passage through rabbits. A typical Strep. pyogenes was used. The results are shown in table 6. The serum of the rabbit immunized with the original streptococcus was agglutinating for the original strain in dilution of 400 but for the streptococcus after 3 and 5 rabbit passages in dilution of 50. The serum 1:400 agglutinated the original culture, but after the rabbit passages agglutination was obtained with a dilution of 1:50 only. On the other hand, the serum of a rabbit injected with the streptococcus after it had been passed through 5 rabbits agglutinated this strain in a dilution of 640, the streptococcus in the original culture and after one rabbit passage in a dilution of 320. It would appear that the streptococcus underwent some change on passage through rabbits.

TABLE 6

AGGLUTINATION BY VARIOUS IMMUNE SERUMS OF STREPTOCOCCUS PASSED THROUGH RABBITS

Immune Serum	Streptococci							
	Original Strain		After 1 Rabbit Passage		After 3 Rabbit Passages		After 5 Rabbit Passages	
	2 Hours	20 Hours	2 Hours	20 Hours	2 Hours	20 Hours	2 Hours	20 Hours
Serum of rabbit injected with original culture....	1:400	1:1600	1:200	1:1600	1:50	1:400	1:50	1:400
Serum of rabbit injected with streptococci after 5 rabbit passages.....	1:160	1:320	1:80	1:320	1:80	1:640	1:80	1:640

Figures give highest active dilution of serum.

Next, the agglutination of streptococci after passage through rabbits, mice and guinea-pigs was studied with the results illustrated by table 7. We see that the streptococci were agglutinated more strongly by the strictly homologous serum. It is notable that the serum of a rabbit injected with streptococci after 5 rabbit passages had less agglutinating power with respect to the streptococci treated in other ways, especially those that had passed through guinea-pigs. The serum produced with a streptococcus that had been passed through 5 rabbits and then through 15 mice agglutinated the homologous streptococci, the streptococci in the original state and the streptococci that had been passed through guinea-pigs, but it had little effect on the streptococci treated in other ways. The serum of rabbits injected with streptococci passed through guinea-pigs agglutinated these cocci more strongly than the cocci that had been passed

through 5 rabbits and 24 mice. The results suggest that the streptococci in the original culture were more like the passage streptococci than these resembled each other.

The normal serum of the guinea-pig, rabbit, goat and horse agglutinated all the various streptococci in low dilution.

TABLE 7

CHANGES IN AGGLUTINABILITY OF STREPTOCOCCUS ON ANIMAL PASSAGE

Immune Serum	Streptococci									
	Original Strain		After 5 Rabbit Passages		After 5 Rabbit and 15 Mouse Passages		After 5 Rabbit and 24 Mouse Passages		After 5 Rabbit, 24 Mouse and 7 Guinea-Pig Passages	
	2 Hours	20 Hours	2 Hours	20 Hours	2 Hours	20 Hours	2 Hours	20 Hours	2 Hours	20 Hours
Serum produced with original strain	1:400	1:1600	1:50	1:400	1:50	1:100	1:50	1:100	1:50	1:100
Serum produced with strain passed through 5 rabbits	1:200	1:800±	1:200	1:1600	1:50	1:200	1:50	1:200	1:50±	1:100±
Serum produced with strain passed through 5 rabbits and 15 mice	1:200	1:800	1:50	1:100	1:400	1:1600	1:50	1:100	1:50	1:100
Serum produced with strain passed through 5 rabbits and 24 mice	1:200	1:800	1:100	1:200	1:50	1:200	1:800	1:1600	1:100	1:800
Serum produced with strain passed through 5 rabbits, 24 mice and 7 guinea-pigs	1:50	1:100	0	1:50	1:50	1:100	1:50	1:200	1:100	1:800

Figures give highest active dilution of serum.

Absorption experiments were made in the following manner: the serum was diluted 10 times with salt solution and 10 loopfuls of the centrifugate of streptococcus cultures in 0.2% dextrose broth added. The suspension was incubated for 2 hours and then centrifugated thoroughly. It was found that when a serum produced by injections of the original streptococcus culture was treated in this manner with this streptococcus strain, all streptococcus agglutinins were removed. After treatment of this serum with streptococci after 5 rabbit and 15 mouse passages, the serum still agglutinated the original streptococcus in a dilution of 1:200, but had no effect on the other streptococcal strains. When the serum was treated with streptococci that had been passed through 5 rabbits, 24 mice and 7 guinea-pigs, it still agglutinated the original streptococci in a dilution of 1:200, and the streptococci that had passed through 5 rabbits in a dilution of 1:100. Furthermore, the treatment of the immune serum produced with streptococci that had passed through 5 rabbits, 24 mice and 7 guinea-pigs with the same streptococcus removed all streptococcus agglutinins, but after treatment with the original streptococcus the serum still agglutinated the strictly homologous streptococcus in a dilution of 1:100 but had no effect on any other streptococci. It may be concluded that the agglutinin are absorbed freely by the streptococci used in the immunization and that the agglutinins that remain vary more or less with respect to their action on related streptococcal strains.

OPSONIFICATION OF STREPTOCOCCI

Table 8 gives the results of phagocytosis experiments with the same serum and streptococcal strains as in the agglutination experiments. Guinea-pig leukocytes were used and the mixtures were incubated for 30 minutes. The results show that the opsonins were increased in all the immune serums and that the virulent strains were more resistant than the less virulent, but there is no indication of any fundamental difference between the different strains.

TABLE 8

OPSONIFICATION OF STREPTOCOCCI BY IMMUNE SERUMS

Immune Serum	Streptococcus in Original Culture	Streptococcus after Rabbit Passages	Streptococcus after Rabbit and 15 Mouse Passages	Streptococcus after Rabbit and 24 Mouse Passages	Streptococcus after Rabbit, 24 Mouse and Guinea-Pig Passages
Serum 1, immune serum produced with original streptococcus	28.4	17.3	8.0	4.0	3.1
Serum 2, immune serum produced with streptococcus after passage through rabbits	29.6	19.0	8.3	4.5	4.5
Serum 3, immune serum produced with passage through mice (24)	27.9	18.3	10.6	9.7	6.2
Serum 4, immune serum produced with streptococcus after passage through rabbits, mice and guinea-pigs	23.1	17.0	12.8	11.1	8.4
Normal rabbit serum	12.0	10.0	0	0	0

PRECIPITATION TESTS

Marmorek[5] showed that specific immune serum may cause precipitate in filtrates of streptococcus cultures. Aronson[6] obtained precipitates with extracts of streptococci in 1% aethylendiamin solution and specific serum, but not with culture filtrate. Eisler[7] obtained precipitates with the concentrated, filtrated and immune serum. Recently Barnes[8] studied the relation between the hemolytic, fermentative and precipitinogenic properties of streptococci, and he concludes that results of the precipitin reaction agree with the results of the hemolytic and fermentative reactions in classifying streptococci. He found, however, that in low dilutions the immune serums would give group reactions with the fluids of streptococcus cultures.

The method I followed was to centrifugate 24-hour cultures in 0.2% dextrose broth, wash the centrifugate with distilled water and then suspend one loopful in 1 c c of water, heat to 60 for one hour, and place in the ice chest for 24 hours. The suspension was then shaken (130 revolutions a minute) for 15 hours and filtered through a maasen filter. Being unable to get definite results with the immune serum used in the agglutination tests, I injected rabbits with the material prepared as just described, beginning with quantities of 1 c c and increasing up to 10 c c, giving the injections intravenously and subcutaneously at intervals of 4-7 days and bleeding 10-12 days after the last injection. In making the test 0.1 c c of immune serum was added to progressive dilutions of the antigen, the total quantity being always made up to 1.1 c c by means of salt solution; the mixtures were then incubated and the result noted at the end of 5, 8 and 24 hours.

[5] Kolle and Wassermann's Handbuch, 1914, 2, p. 787.

[6] Berl. klin. Wchnschr., 1902, p. 979.

[7] Kolle and Wassermann's Handbuch, 1914, 2, p. 784.

[8] Jour. Infect. Dis., 1919, 25, p. 47.

It was found that immune serum was strongly precipitative for the streptococci used in producing the serum and reacted weakly with all the other strains. The nature of the precipitinogen in streptococci apparently changes under different conditions.

ACID AGGLUTINATION OF STREPTOCOCCI

According to Michaelis[9] acid agglutination depends on the precipitability of proteins by hydrogen ions. In order to study this form of agglutination of streptococci the following solutions were prepared, each containing normal NaOH solution 5 c c and increasing quantities of normal HCl solution as shown below, the total quantity in each case being made 100 c c by the addition of distilled water:

[9] Deutsche med. Wchnschr., 1911, 37, p. 969.

Solutions	Normal HCl Solution	Quantity of H-ion
1	7.5	1.10-5
2	10	2.5-5
3	15	4.10-5
4	25	8.10-5
5	45	16.10-5
6	85	30.10-5

The centrifugated sediment of 24-hour cultures in 0.2% glucose broth were washed thoroughly with distilled water and finally suspended in distilled water to which a small amount of cinnobar was added. After agitation and standing for a few hours the suspensions were centrifugated a short time in order to remove the cinnobar. In each case 1 c c of streptococcus suspension was mixed with acid solution and incubated for one and a half hours. As shown in table 9, there was little difference in the agglutination of the various strains of streptococci treated in this way.

TABLE 9

ACID AGGLUTINATION OF STREPTOCOCCI

Streptococcal Strains	Acid Solutions						
	1	2	3	4	5	6	Distilled Water
Original streptococcus	++	+	+	+	+	±	0
After rabbit passages	+	++	+	+	+	0	0
After rabbit and mice passages	+	++	+	±	0	0	0
After rabbit, mice and guinea-pig passages	+	+	++	+	+	0	0
After 100 mice passages	+	++	+	0	0	0	0
Staphylococcus aureus	+	+	+	++	+	0	0
Pneumococcus	0	0	0	0	0	0	0

CONGLUTINATION OF STREPTOCOCCI

Bordet and Gay[10] found that beef serum, heated to 56 C., would cause clumping and increased lysis of blood corpuscles in the presence of inactivated hemolytic serum and complement. Later Bordet and Streng[11] called the sub-

[10] Ann. de l'Inst. Pasteur, 1906, 20, p. 467.

[11] Centralbl. f. Bakteriol., I. O., 1909, 47, p. 260.

stance in the beef serum causing this action conglutinin and Streng found that it acts not only on corpuscles but also on bacteria; furthermore, that it is active in the presence of specific antiserum from which agglutinins have been removed by absorption. The method has been used with some success to differentiate between certain closely related bacteria, but Swift and Thro[12] found that in the case of streptococci conglutination was not of any greater value than ordinary agglutination.

In my experiments I used the same streptococcal suspensions and immune serum (inactivated) as in the agglutination tests. Guinea-pig serum was used as complement, 0.5 c c of a 10% dilution in salt solution were used in each test. Fresh beef serum, after being heated to 56 C. for 30 minutes, was added to each mixture to the amount of 0.5 c c of a 10% dilution in salt solution. The mixtures were incubated for 2 hours. All the reactions were strongest as a rule in the serum mixtures containing the homologous streptococcus.

TABLE 10

CONGLUTINATION AND AGGLUTINATION OF STREPTOCOCCI BY IMMUNE SERUMS

Streptococcal Strains	Immune Serums			
	Serum 1	Serum 2	Serum 3	Serum 4
1. Original streptococcus:				
Conglutination	0.0005	0.001	0.001	0.005
Agglutination	0.0025	0.005	0.005	0.025
2. Streptococcus after rabbit passages:				
Conglutination	0.0005	0.0005	0.025	0.025
Agglutination	0.0025	0.001	0.025	0.025
3. Streptococcus after rabbit and mouse passages:				
Conglutination	0.005	0.01	0.0005	0.01
Agglutination	0.005	0.01	0.0025	0.01
4. Streptococcus after rabbit, mouse (24) and guinea-pig passages:				
Conglutination	0.005	0.025	0.005	0.001
Agglutination	0.001	0.025	0.005	0.0025
Streptococcus 0:				
Conglutination	0.025	0.025	0.025	0.025
Agglutination	0.025	0.025	0.025	0.025
Staphylococcus:				
Conglutination	0	0	0	0
Agglutination	0	0	0	0
Pneumococcus:				
Conglutination	0	0	0	0
Agglutination	0	0	0	0

The figures give the smallest amount of serum causing conglutination and agglutination.

COMPLEMENT FIXATION TESTS

The various strains of streptococci obtained after passages through different animals were used in complement fixation tests in order to determine, if possible, whether there was any difference in their antigen properties. Antisheep rabbit serum was used, the titer being 0.0005 and double this dose was used in the tests. The antigen consisted of the washed centrifugate of 24-hour streptococcus cultures in 0.2% dextrose broth, suspended in distilled water, then heated at 55 C. for one hour and thoroughly shaken in a machine, many small glass balls first being added. The suspension was then centrifugated and the supernatant fluid mixed with 10% salt solution, making a suspension in 0.9% salt solution. The dose of the antigen in the fixation test was 0.5 c c. The same antistreptococcus serum was used as in the agglutinin experiments. The results revealed that no difference between the different streptococcus cultures could be made out.

[12] Arch. Int. Med., 1911, 7, p. 24.

SUMMARY

The virulence of a streptococus rapidly falls on artificial cultivation, particularly on blood agar. The amount of peptone in the medium does not seem to influence the virulence so much as the reaction, acid reaction maintaining virulence better than alkaline. The virulence persists longer in anaerobic than in aerobic conditions.

A streptococcus that has been cultivated artificially for some time and has become avirulent increases in virulence for both rabbits and mice on passage through the rabbit. If also passed through mice, the virulence is further increased, especially for mice, and when a certain maximum in virulence has been reached no further increase develops on further passages through mice.

When maximum virulence for mice has been established, passage through rabbits may increase the virulence for rabbits but decrease it for mice. On the other hand, if virulence for mice is still on the increase, passage through the rabbit may increase the virulence for both rabbits and mice.

Virulence may be increased by keeeping streptococci in a collodion sac in the peritoneal cavity of rabbits.

The agglutinability of a streptococcus may change as the result of animal passage, the particular strain used for immunization being agglutinated more strongly than the related strains by the corresponding immune serum. The original nonvirulent mother streptococcus was agglutinated by all the immune serums. The same relation seems to obtain with reference to opsonins and phagocytosis, as well as with respect to specific precipitation, and conglutination, but no differences could be made out between the different strains by means of complement fixation.

All the various strains were agglutinated in the same way by acid solution.